Two new species of the genus *Euphlyctis* (Anura, Ranidae) from southwestern India, revealed by molecular and morphological comparisons

S. Hareesh JOSHY *, Mohammad Shafiqul ALAM **, Atsushi KURABAYASHI **, Masayuki SUMIDA ** & Mitsuru KURAMOTO ***

* Rondano Biodiversity Research Laboratory, St. Aloysius College, Mangalore 575 003, Karnataka, India

** Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Higashihiroshima, Hiroshima 739-8526, Japan

3-6-15 Hikarigaoka, Munakata, Fukuoka 811-3403, Japan

Two new frog species of the genus *Euphlyctis*, which were shown to be two distinct taxa by mitochondrial DNA analyses, are described from Karnataka State, southwestern India. On the molecular phylogenetic tree, the first new species appears as a sister group with respect to *E. hexadactylus*. The second new species forms a group with *E. cyanophlyctis*. The first species differs from *E. hexadactylus* in having a distinctly smaller snout-vent length and dark brown bold markings on the dorsum, a smaller head, shorter hindlimbs and wider eyelids, relative to snout-vent length. The second species differs from the close relative *E. cyanophlyctis* in having shorter fingers. Its advertisement calls are composed of trills that are much longer in duration, are composed of more numerous pulses, and have a lower dominant frequency than those of *E. cyanophlyctis* and *E. hexadactylus*. Morphological comparisons between the four species are presented. The present study reveals hitherto overlooked cryptic biodiversity in the genus *Euphlyctis*.

INTRODUCTION

Euphlyctis is a small genus comprising only four currently recognized species: *E. cyanophlyctis* (Schneider, 1799) from Iran, Afghanistan, Pakistan, Nepal, India, Sri Lanka, Malaya and Vietnam, *E. ehrenbergii* (Peters, 1863) from Saudi Arabia and Yemen, *E. ghoshi* (Chanda, 1991) from Manipur, India, and *E. hexadactylus* (Lesson, 1834) from India, Sri Lanka and Bangladesh (FROST, 1985; CHANDA, 1991; DUBOIS, 1992). *Euphlyctis cyanophlyctis* and *E. hexadactylus* are known to occur in southwestern India (BIJU, 2001; DANIELS, 2005). These species are aquatic or semi-aquatic frogs with wide toe webbing that usually live half-submerged in water, or on the water edge of ponds, wetlands, paddy fields and ditches.

In 2003, we collected small frogs of the genus *Euphlyctis* from Mangalore, together with *E. hexadactylus* and *E. cyanophlyctis*. At first, we considered the small ones as juveniles of *E. hexadactylus*. However, mtDNA data revealed that the small frogs were distinctly different from *E. hexadactylus* as well as from *E. cyanophlyctis* (KURABAYASHI et al., 2005; ALAM et al., 2008). We collected similar small *Euphlyctis* frogs from Mudigere in the Western Ghats in 2007, and the mtDNA data, described in the present study, clarified that the frogs from Mudigere differed from those of Mangalore. ALAM et al. (2008) also demonstrated the presence of another cryptic *Euphlyctis* species from Bangladesh by mtDNA analysis, but the two new Indian taxa here treated were clearly different from that from Bangladesh. These latter two Indian frogs are described below as two new species.

Recently, many new anuran species have been described from southwestern India, including the Western Ghats (e.g., DUBOIS et al., 2001, BIJU & BOSSUYT, 2003, 2005, 2006, KURAMOTO & JOSHY, 2003; BIJU et al., 2007; KURAMOTO et al., 2007). This indicates that the wealth of amphibian biodiversity in this area is beyond the expectation generally recognized. The present study and other recently obtained evidence sheds light on the cryptic biodiversity in the small and rather unnoticed genus *Euphlyctis*.

MATERIAL AND METHODS

Euphlyctis frogs were collected from Adyar (12°52'N, 74°55'E, altitude 1 m) and Bajpe (12°58'N, 74°50'E, altitude ca. 70 m) in Mangalore, Dakshin Kannad District of Karnataka, and from Mudigere (13°07'N, 75°31'E, altitude ca. 1020 m), Chikumagalur District of Karnataka, during the rainy season (May to July), from 2003 to 2008. To elucidate the genetic divergence and phylogenetic relationship of the *Euphlyctis* taxa occurring in southwestern Karnataka, partial mtDNA portions corresponding to 12S and 16S rRNA genes were analyzed for 37 *Euphlyctis* samples involving those of *E. hexadactylus* from Adyar and *E. cyanophlyctis* from Bajpe, Padil (Mangalore), Karnoor (Dakshin Kannad District) and Madikeri (Kodagu District).

In the present study, the mtDNA fragments were newly amplified and sequenced for 14 specimens and the data of the remaining 23 taxa were obtained from our previous studies (ALAM et al., 2008). The DNA amplification and sequence strategies followed the procedures as in the previous papers. The resultant sequences of each 12S and 16S rRNA gene were initially aligned using ClustalX 1.83 (THOMPSON et al., 1997). the initial 12S and 16S rRNA alignment data contained 566 and 520 nucleotide sites, respectively. From these alignment data, the genetic divergence (uncollected *p* value) between taxa was calculated. To perform sophisticated phylogenetic analyses, gaps and ambiguous alignment sites were excluded from the initial alignments using Gblocks 0.91b (CASTRESANA, 2000). To check whether 12S and 16S rRNA data could be submitted to combined analyses, a permutation homology test (FARRIS et al., 1995) was conducted using PAUP* 4.10b (SWOFFORD, 2001) ($P = 0.124$). Then, the two gene data were concatenated. The concatenated alignment data contained a total of 976 nucleotide sites, 192 of which were parsimoniously informative. Phylogenetic analyses based on the concatenated data were conducted sing maximum likelihood (ML) and Bayesian inference (BI) methods. In these analyses, *Fejervarya limnocharis* (accession no. AY158705; LIU et al., 2005) and *Limnonectes fujianensis* (AY974191, NIE et al., unpublished) were used as outgroups. For ML and BI analyses, appropriate substitution models were estimated using Akaike information criteria implemented in Modeltest 3.7 (POSADA & CRANDALL, 1998), and a general time-reversible substitution model with gamma population and proportion of invariable sites sub-models (GTR+G+I) was chosen. ML analysis was performed using PAUP*. Nonparametric bootstrap (BP) values under ML were calculated with 300 replicates. BI analysis was performed using MrBayes 3.1.2 (RONQUIST & HUELSENBECK, 2003). The following settings were also used for the BI analysis: number of Markov chain Monte Carlo generations = 15×10^5 and sampling frequency = 10. The burn-in size was determined by checking convergences of –log likelihood (–ln*L*) values, and the first 1×10^5 generations were discarded. The statistical support of the resultant BI tree was evaluated by Bayesian posterior probabilities (BPP).

Measurements were recorded for snout-vent length (SVL), head length (HL), head width (HW), snout to nostril distance (S-N), inter-nostril distance (N-N), nostril to eye distance (N-E), eye diameter (ED), inter-orbital distance (E-E), eyelid width (ELW), tympanum diameter (TD), hand length (HAL), no. 1 to no. 4 finger length (F1-F4), hindlimb length (HLL), femur length (FEL), tibia length (TIL), foot length (FOL), and no. 1 to no. 5 toe length (T1-T5). For details of the method of measurements see KURAMOTO & JOSHY (2006) and KURAMOTO et al. (2007). Juvenile specimens were excluded from measurements. For morphological comparison, we measured six preserved specimens of *E. hexadactylus* from Adyar, Mangalore and 19 specimens of *E. cyanophlyctis* from Mangalore, Karnoor, Bhatkal, Talagini, Mudigere and Madikeri, all in Karnataka State (see fig. 1 in KURAMOTO et al., 2007), deposited in the Rondano Biodiversity Research Laboratory, St. Aloysius College. Examined specimens are listed below except for those of the new species. Discriminant analyses were performed by SPSS (15.0J) statistics software (SPSS Japan, Inc.) using the measurements without any transformation.

Euphlyctis cyanophlyctis. Bajpe: RBRL 04070611, 05072202, 07072114 (1 adult ♂, 2 adult ♀). Bhatkal: RBRL 00062601-00062603, 00062605-00062607 (6 adult ♀). Karnoor: RBRL 01080508, 04071139, 04071140 (2 adult ♂, 1 adult ♀). Madikeri: RBRL 03060702 (1 adult ♀). Mudigere: RBRL 05070921, 05070922 (1 adult ♂, 1 adult ♀). Padil: RBRL 03052303 (1 adult ♀). Talagini: RBRL 01081113, 01081114, 01081118 (3 adult ♀).

Euphlyctis hexadactylus. Adyar: RBRL 03060601, 05071901-05071903, 07072801, 07072802 (5 adult ♂, 1 adult ♀).

The advertisement calls were recorded in Mudigere on 29 July 2007 at an air temperature of 23.2°C and on 27 July 2008 at 21.0°C using an MD recorder (Sony MZ-B10). The recorded calls were analyzed by Avisoft-SASLab Light software (Avisoft Bioacoustics).

The type specimens were deposited in the Natural History Collections of the Bombay Natural History Society (BNHS), and the other specimens were stored in the Rondano Biodiversity Research Laboratory, St. Aloysius College (RBRL).

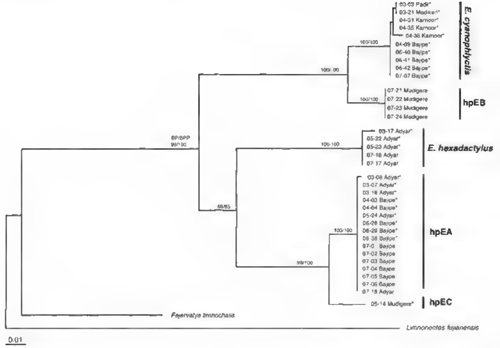


Fig. 1. - Phylogenetic relationships of *Euphlyctis* taxa from Karnataka, India, inferred from mitochondrial 12S and 16S rRNA gene data. Maximum likelihood tree (–ln*L* = 3356.93) is represented here. Bayesian analysis reconstructed the same tree topology. The numbers on the nodes are BP in ML and BPP in BI. Three haplotype groups are shown by abbreviations, hpEA, hpEB and hpEC. Field numbers of samples and collecting sites are shown. Asterisks indicate that the samples were used in analyses by Kurabayashi et al. (2005) and Alam et al. (2008).

Results

Molecular phylogeny and genetic divergence of the *Euphlyctis* taxa from Karnataka

Based on the 12S and 16S rRNA gene sequences, the Indian *Euphlyctis* specimens consisted of five major haplotype groups (fig. 1). Two of the five groups corresponded to *E. cyanophlyctis* and *E. hexadactylus*, and the others were temporarily named as hpEA, hpEB and hpEC. In the ML tree (fig. 1), the hpEB group formed a group with *E. cyanophlyctis* and this clade was strongly supported by statistical values (BP = 100; BPP = 100). The hpEA and hpEC groups formed a group, and they became a sister taxon with respect to *E. hexadactylus*, but statistical support for this relationship was not high (BP = 68, BPP = 85). The same relationships as for the five major *Euphlyctis* taxa were also reconstructed in our Bayesian analysis. Furthermore, the present result was partially congruent with the results of previous studies. Kurabayashi et al. (2005) showed that small-sized *Euphlyctis* specimens (hpEA) rom Mangalore (Adyar and Bajpe) differed genetically from *E. hexadactylus*, and ALAM et al 2008) found that one specimen from Mudigere (hpEC) was closely related to the hpEA roup, but there was a degree of genetic divergence between the groups.

According to ALAM et al (2008), the average sequence divergences between *E. hexadactylus* and hpEA (Ehex-In1 and Ehex-In2 in ALAM et al., 2008) were 11.9 % and 6.3 % for 12S nd 16S rRNA genes, respectively Because these values were larger than those previously eported from intraspecific sequence comparisons in mantellids (VENCES et al., 2005) and outh American bufonids and hylids (FOUQUET et al., 2007), ALAM et al (2008) concluded iat the two haplotype groups should be separated taxonomically as different species. When e recalculated the average sequence divergence between these taxa with the present addition-l material, the values were 13 0 % and 9.1 % for 12S and 16S rRNA genes, respectively. The pecimen from Mudigere collected in 2003 (hpEC, Ehex-In3 in ALAM et al., 2008) was also eparated clearly from *E hexadactylus* (15 3 % and 9 1 % for 12S and 16S), but the sequence livergence values (5 0 % and 2 3 %) did not support the distinct separation between the hpEC nd hpEA groups. Only one specimen with the hpEC haplotype has been found so far, and his specimen was apparently subadult Thus, more specimens are needed before discussing its axonomic status.

The most remarkable finding in the present study was that the five specimens from Mudigere (hpEB) collected in 2007 formed a sister group to that of *E cyanophlyctis* (fig. 1). Molecular divergence between hpEB and *E cyanophlyctis* was 16.4 % for 12S and 10 7 % for 16S rRNA genes. As in the case between hpEA and *E. hexadactylus*, these values were large nough to regard the hpEB group as a distinct species from *E cyanophlyctis*.

Our molecular analyses have revealed the occurrence of two undescribed species in southwestern part of Karnataka. As discussed in the later section, the two haplotype (hpEA and hpEB) groups were morphologically distinct from *E hexadactylus* and *E cyanophlyctis*, espectively, and from each other These indicate that the two haplotype groups are reproductively distinct, and are described below as new species.

TAXONOMY

Euphlyctis aloysii sp. nov.
(fig. 2-3)

hpEA group in fig. 1 and in KURABAYASHI et al. (2005).
Ehex-In2 group in ALAM et al. (2008)

Diagnosis Small *Euphlyctis* species, SVL from 31 8 to 45 2 mm in females. It differs from *E hexadactylus* in its distinctly smaller body size, having four large elliptical dark markings on the dorsum, smaller head, shorter hindlimbs, and wider eyelids, relative to SVL. The presence of large dorsal markings and thin mid-dorsal stripe readily distinguishes this species from *E. cyanophlyctis* The eyes and tympanums are smaller, and femur and tibia are shorter, relative to SVL, in *E. aloysii* than in *E cyanophlyctis*

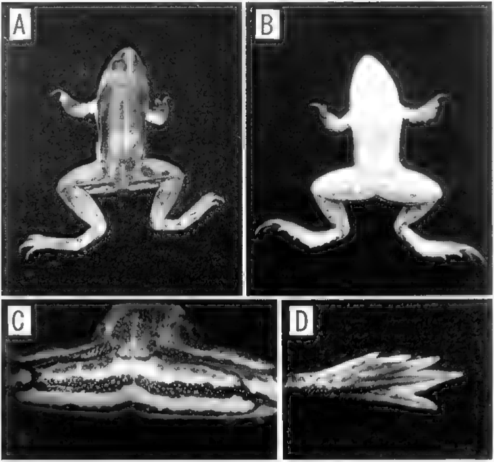


Fig. 2. – Holotype of *Euphlyctis aloysii* sp. nov. (BNHS 5123, ♀ from Bajpe). Dorsal view (A), ventral view (B), posterior aspect of thigh (C), and foot (D). Lower part of abdomen was cut open for sexing, and the opening is seen in B.

Holotype. – BNHS 5123 (fig. 2), female, SVL 40.4 mm, collected in Bajpe, Mangalore, on 21 July 2007.

Paratypes. – BNHS 5124, ♀, SVL 38.6 mm, Adyar, Mangalore, 6 June 2003; BNHS 5125, ♀, SVL 37.1 mm, Bajpe, Mangalore, 21 July 2007; BNHS 5126, ♀, SVL 37.2 mm, Adyar, Mangalore, 28 July 2007.

Other specimens examined. – RBRL 03052501, 05071904, two adult ♀, Adyar. RBRL 04070601-04070603, 06072003-06072004, 06072404, 07072101, 07072104-07072113, 07072115, 18 adult ♀, Bajpe.

Description of holotype (measurements in mm). – Vomerine teeth round, situated near anterior end of upper jaw; tongue tip bifurcated.

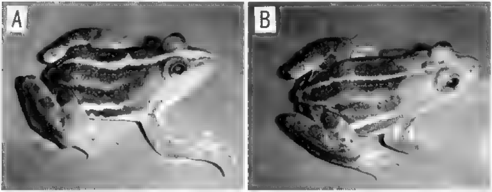


Fig. 3. *Euphlyctis aloysii* sp. nov. RBRL 06072004 (A) and RBRL 06072404 (B), showing coloration in life.

Head small, wider than long (HL 12.4, HW 13.1); snout slightly pointed; nostril nearer to tip of snout than to eye (S-N 2.9, N-E 3.1), loreal region concave, canthus rostralis blunt; internarial distance larger than inter-orbital, the latter smaller than eyelid width (N-N 2.4, E-E 1.4, ELW 3.3); tympanum large, about 75 % of eye diameter (ED 4.2, TD 3.3).

Finger free, finger tip small, slightly pointed; first finger longer than second (F1 7.0, F2 4.5); subarticular tubercle moderate, finger lengths F2 < F4 < F1<F3 (F3 7.2, F4 4.7).

Distal part of thigh thick; tibio-tarsal articulation slightly apart when legs folded at right angle to body axis, foot length larger than femur length and slightly larger than tibia length (FOL 19.1, FEL 18.4, TIL 19.0); toe tip small, slightly pointed; subarticular tubercle moderate, toe lengths T1 < T2 < T3 < T5 < T4 (T1 7.1, T2 9.9, T3 11.8, T4 15.6, T5 13.4); web nearly reaching toe tip and sharply incised (fig. 2D); inner metatarsal tubercle indistinct.

Supra-tympanic fold thin, forming granular row at posterior part of tympanum, not reaching arm base, numerous small round ridges on dorsum, no ridges on flank and thigh, underside smooth, except a pair of rows consisting of a series of small dermal projections from the anterior edge of forelimbs to groin.

In preservative, dark brown above with a thin mid-dorsal stripe, small black spots from beneath eye to forelimb base; large dark brown elliptical or round markings on dorsal side of thigh and shank, wide white longitudinal stripe on sides from above forelimb to groin; three dark brown longitudinal stripes and intervening two white stripes on posterior side of thigh (fig. 2C), thin pale stripe on outer edge of shank, dark streak from ankle to outer edge of foot; ventral side white; irregular dark line pattern on underside of thigh (fig. 2B), irregular dark markings on underside of shank.

Color in life. Dorsum light brown with a thin greenish mid-dorsal stripe, and green patches over upper jaw and from eyelid to shoulder, two pairs of rather conspicuous large elliptical markings on dorsum (fig. 3). At night, the dorsum was darker, and green color and dorsal markings became inconspicuous.

Variation – Measurements for 24 female specimens are given in tab. 1 Of 24 specimens, 22 had a thin mid-dorsal stripe (fig. 3B), one had a relatively thick mid-dorsal stripe (fig. 3A), and only one (paratype BNHS 5124) lacked mid-dorsal stripe. Irregular line pattern on underside of thigh and shank differed from specimen to specimen, and extended to lower part of abdomen in some specimens. Paratype BNHS 5124 showed a distinct black dot line system composed of black horny tubercles; a curved dot line between anterior edge of foreleg, a pair of dot lines on both sides of the throat, a pair of dotted lines from the anterior part of the arm base, circling the upper edge of arm base, extending toward groin, then toward back; a pair of faint longitudinal black dotted lines on both sides of the venter A similar dotted line system was reported in *E cyanophlyctis* from Sri Lanka (DUTTA & MANAMENDRA-ARACHCHI, 1996), and one of the authors (MK) observed it in a preserved specimen of *E hexadactylus* from Malabar (deposited in Muséum national d'Histoire naturelle, Paris: MNHN 1292.9, SVL 69 2 mm) These systems apparently represent the lateral line system (see DUBOIS & OHLER, 2001).

We did not observe juveniles of *E. hexadactylus*. The juveniles were described as "beautifully striped" (BOULENGER, 1890), "have bars or spots of dark green and black on the back" (DANIEL, 2002), or "more strikingly colored with patches of green and black scattered over the olive-black back" (DANIELS, 2005). These descriptions fit the coloration of *E aloysii* fairly well. Although precise comparisons wait for future studies, there may be a possibility that *E aloysii* has been confused with juveniles of *E. hexadactylus* in some cases. The juveniles of *Hoplobatrachus tigerinus* have a beautiful green and black dorsal pattern, but they can be readily distinguishable from *E. aloysii* by the presence of many longitudinal dermal ridges on the back

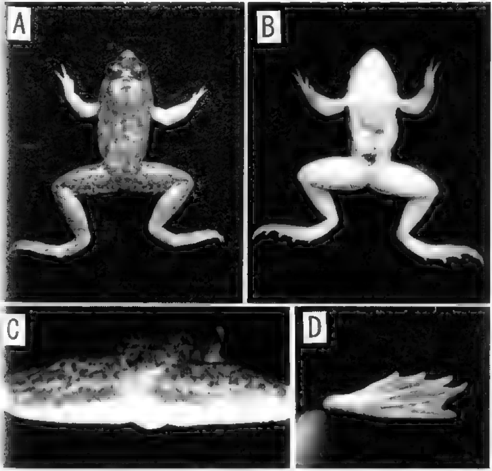
Our specimens were all females, and male sexual characters are unknown

Ecology Females had mature ova in the ovaries. The ova are pigmented and ca. 1 mm in diameter Since the gravid females were collected from late May to late July, spawning may begin in early August. During July, in the middle of the rainy season in Karnataka, we heard advertisement calls of *E hexadactylus*, *Fejervarya caperata* Kuramoto et al., 2007 and *Hylarana aurantiaca* (Boulenger, 1904) in Adyar and those of *Fejervarya caperata*, *F sahyadris* (Dubois et al , 2001), *Microhyla ornata* (Dumeril & Bibron, 1841) and *Polypedates maculatus* (Gray, 1830) in Bajpe, but we could not hear the calls of *E aloysii* Our specimens (n – 24) were composed of females only The reason why males did not appear during our collecting was not clear

Distribution Presently known only from Adyar and Bajpe in Mangalore. The hpEC group from Mudigere, which apparently relates to *E aloysii* from external morphology and molecular analysis may suggest the presence of a montane subspecies.

Etymology This species and the College where the main part of this study was carried out, were both named in honor of Aloysius Gonzaga (1568 1591) Aloysius was a Prince in Italy who entered a Jesuit order and died serving the plague-stricken people of Rome

DNA sequence data for holotype. Accession numbers are AB273171 and AB272606 for mitochondrial 12S and 16S rRNA genes, respectively (07-02 in fig 1)



ig. 4 Holotype of *Euphlyctis mudigere* sp. nov. (BNHS 5127, ♂ from Mudigere). Dorsal view (A), ventral view (B), posterior aspect of thigh (C), and foot (D). Opening for removing tissue for DNA analysis is seen in B.

Euphlyctis mudigere sp. nov.
(fig. 4-6)

pEB group in fig. 1

Diagnosis. — Small *Euphlyctis* species with SVL from 28.1 to 34.8 mm in males. It differs from *hexadactylus* and *E. aloysii* in having a simple stripe pattern on the posterior side of the thigh and a bluntly incised web. The fingers, relative to SVL, are shorter than in *E. cyanophlyctis*. The advertisement calls are 1.3 s in mean duration, and consist of about 16 pulses with the dominant frequency band at about 1.5 kHz. The calls differ from those of *E. cyanophlyctis* and *E. hexadactylus*: call length longer, more numerous pulses in a call and lower dominant frequency band.

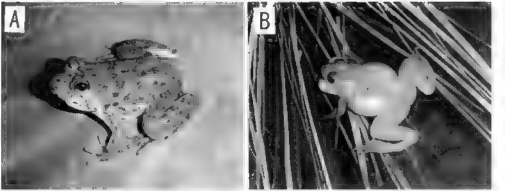


Fig. 5. – *Euphlyctis mudigere* sp. nov. Paratype (BNHS 5130) (A) and RBRL 08072504 (B) showing coloration in life.

Holotype. – BNHS 5127 (fig. 4), male, SVL: 31.1 mm, collected in Mudigere, on 29 July 2007.

Paratypes. – BNHS 5128, ♂, SVL 29.2 mm, Mudigere, 29 July 2007. BNHS 5129, ♂, SVL 28.1 mm, Mudigere, 29 July 2007. BNHS 5130 (fig. 5A), ♂, SVL 32.7 mm, Mudigere, 29 July 2007.

Other specimens examined. – RBRL 07072905, 08072504 (fig. 5B), 08072505, three ♂, Mudigere.

Description of holotype (measurements in mm). – Vomerine teeth round, situated near anterior end of upper jaw; tongue tip bifurcated.

Head small, wider than long (HL 10.3, HW 11.3); snout slightly pointed; nostril nearer to eye than to tip of snout (S-N 3.0, N-E 2.6); loreal region concave, canthus rostralis blunt, internarial distance larger than inter-orbital, the latter smaller than eyelid width (N-N 2.1, E-E 1.2, ELW 2.3); tympanum large, about 85 % of eye diameter (ED 3.8, TD 3.3).

Fingers free, gradually tapering to pointed tip; first finger larger than second (F1 4.6, F2 3.9), subarticular tubercle small; finger lengths F4 < F2 < F1 < F3 (F3 5.6, F4, 3.5). No thickening of the first finger, corresponding to nuptial pad, was noticed.

Distal part of thigh thick, tibio-tarsal articulation slightly apart when legs folded at right angle to body axis, femur length larger than tibia length, the latter larger than foot length (FEL 15.6, TIL 14.2, FOL 13.8), toe tip small, slightly pointed; subarticular tubercle small; toe lengths T1 < T2 < T5 < T3 < T4 (T1 5.1, T2 7.4, T3 10.3, T4 11.5, T5 10.1), web large, nearly reaching toe tip and bluntly incised (fig. 4D), inner metatarsal tubercle indistinct.

Dorsal surface with small tubercles; supra-tympanic fold present, but not distinct, underside smooth. A pair of vocal sacs on both sides of lower jaw near jaw angle.

In preservative, dorsum dark brown with indistinct small patches, irregular markings on upper side of hindlimb, a conspicuous white band on posterior side of thigh, accompanied with a thin black stripe on ventro-posterior side (fig. 4C), no mid dorsal stripe, underside immaculate, vocal sacs light gray.

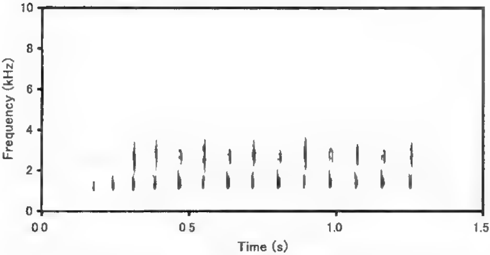


Fig. 6. – Sound spectrogram of the advertisement call of *E. mudigere* sp. nov. (FlatTop window, 323 Hz bandwidth).

Color in life. – Dorsum was light brown with many small darker patches (fig. 5A). In the night, these patches tended to fade (fig. 5B).

Variation. – Measurements for seven male specimens are given in tab. 1. None of the specimens had a mid-dorsal stripe. In external morphology, no distinct intra-specific variation was noticed. Because only male specimens were available, sexual variation is not known.

Advertisement calls. – The advertisement calls of *E. mudigere* recorded on 29 July 2007 at 23.2°C (fig. 6) were trills composed of 16.39 ± 2.77 pulses (n = 18, range 11 - 22), with total length of 1.31 ± 0.22 s (0.84 – 1.71 s). Pulse repetition rate was 11.71 ± 0.56 pulse/s. Frequencies were rather continuous from 1 to over 8 kHz. The dominant and fundamental frequency was at about 1.5 kHz and a second harmonics band was noticed at about 3 kHz. The calls recorded on 27 July 2008 at 21.0°C were nearly the same in number of pulses (16.36 ± 1.92 pulses, range 12 – 20, n = 22), but the call length was longer (1.48 ± 0.21 s, range 1.05 - 1.92 s) and the pulse repetition rate was lower (11.10 ± 0.32 pulse/s) than the calls recorded in 2007. The differences between the two recordings in call length and pulse repetition rate were slight, but statistically significant (t = 2.428 and P = 0.020 for call length, t = 4.317 and P = 0.0001 for pulse repetition rate). Because the call length became shorter and pulse repetition rate became higher with increasing temperatures (e.g. KURAMOTO & JOSHY, 2006), these may be due to the slight difference in air temperature at the time of recordings.

The advertisement calls of *E. cyanophlyctis* and *E. hexadactylus* were analyzed by KURAMOTO & JOSHY (in press). The calls of *E. mudigere* differed from the calls of *E. cyanophlyctis* which were not the trills but typically composed of a series of two-pulse notes. Compared with the calls of *E. mudigere*, the calls of *E. hexadactylus* were shorter in call duration (0.25 ± 0.07 s), fewer in pulse number (5.0 ± 1.18) and higher in dominant frequency (2.29 – 2.43 kHz).

Table 1. – Mean ($\bar{x}$), standard deviation (s) and range in measurements (in mm) of four *Euphlyctis* species from Karnataka, India. See text for character abbreviations.

Measurement	*E. aloysii*		*E. mudigere*		*E. cyanophlyctis*				*E. hexadactylus*		
	Females (n = 24)		Males (n = [illegible])		Females (n = 15)		Males (n = 4)		Female	Male (n = [illegible])	
	$\bar{x} \pm s$	min - max	$\bar{x} \pm s$	min - max	$\bar{x} \pm s$	min - max	$\bar{x} \pm s$	min - max	(n = 1)	$\bar{x} \pm s$	min - max
SVL	37.58 ± 3.[illegible]5	31.8 - 45.2	31.2 ± 2.25	28.1 - 34.8	42.30 ± 7.01	33.0 - 56.9	35.10 ± 3.43	30.9 - 38.1	88.5	62.70 ± 4.32	56.0 - 67.7
HL	10.85 ± 0.98	9.2 - 12.5	9.53 ± 1.27	7.8 - 11.2	13.09 ± 2.[illegible]2	10.8 - 18.1	1[illegible].08 ± 1.13	9.6 - 12.1	28.7	22.[illegible] ± 2.88	19.1 - 25.4
HW	12.38 ± 1.00	[illegible]0.8 - 14.3	11.2 ± 0.88	10.3 - 12.2	14.21 ± 2.81	11.2 - 2[illegible].0	12.00 ± 1.30	10.7 - [illegible]3.4	31.1	22.30 ± 1.72	20.0 - 24.5
S-N	2.40 ± 0.36	1.8 - 3.4	2.43 ± 0.71	1.5 - 3.3	3.[illegible]5 ± 0.66	2.0 - 4.2	2.53 ± 0.6[illegible]	1.9 - 3.3	5.7	5.26 ± 0.59	4.5 - 5.9
N-N	2.47 ± 0.30	2.0 - 3.1	2.19 ± 0.20	1.9 - 2.5	2.45 ± 0.49	1.7 - 3.6	2.58 ± 0.25	2.1 - 2.7	3.5	3.26 ± 0.1[illegible]	3.1 - 3.4
N-E	2.64 ± 0.37	1.9 - 3.7	2.49 ± 0.94	1.6 - 4.2	3.45 ± 0.68	2.3 - 5.0	2.45 ± 0.72	1.8 - 3.3	8.5	5.74 ± 0.42	5.1 - 6.2
ED	3.86 ± 0.56	2.3 - 5.0	[illegible].86 ± 0.74	2.5 - 4.9	5.34 ± 0.89	3.9 - 6.6	4.65 ± 0.52	3.9 - 5.0	7.7	7.[illegible]8 ± 0.66	6.2 - 8.0
E-E	1.62 ± 0.29	1.2 - 2.3	1.49 ± 0.25	1.2 - 1.9	1.83 ± 0.45	1.[illegible] - 2.6	2.00 ± 1.48	1.0 - 4.2	3.4	2.[illegible]8 ± 0.48	[illegible] - [illegible]
ELW	2.50 ± 0.29	1.9 - 3.3	2.1[illegible] ± 0.34	1.4 - 2.5	2.69 ± 0.68	2.0 - 4.3	2.20 ± 0.58	1.4 - 2.8	4.8	3.52 ± 0.54	[illegible]
TD	3.45 ± 0.50	2.6 - 4.5	3.0[illegible] ± 0.89	1.4 - 4.0	4.63 ± 0.82	3.3 - 5.9	4.15 ± 0.74	3.1 - 4.8	6.6	6.26 ± 0.63	5.5 - [illegible]
IAL	9.04 ± 0.96	6.3 - [illegible]0.8	6.97 ± [illegible]	5.0 - 9.5	10.[illegible] ± [illegible].78	8.6 - 14.7	9.58 ± 0.60	9.0 - [illegible]0.4	14.6	14.52 ± 1.15	13.3 - [illegible]6.[illegible]
F1	5.49 ± 1.00	4.2 - 7.4	4.83 ± 0.87	3.8 - 6.1	8.29 ± [illegible].61	6.4 - 11.9	6.[illegible]5 ± 0.93	5.5 - 7.5	13.6	9.78 ± 1.[illegible]	8.2 - 11.6
F2	4.52 ± 0.53	3.5 - 5.6	3.89 ± 0.58	3.0 - 4.6	6.[illegible]8 ± [illegible]	4.[illegible] - [illegible]8	6.08 ± 1.19	5.1 - 7.8	15.9	8.44 ± 0.82	7.8 - 9.8
F3	6.44 ± 0.76	4.8 - 7.7	5.79 ± 0.68	4.8 - 6.9	7.71 ± [illegible].33	6.0 - 10.[illegible]	6.93 ± 0.70	6.0 - 7.6	17.1	1[illegible].38 ± 1.03	9.9 - 12.[illegible]
F4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
HLL	52.95 ± 5.09	44.7 - 64.7	45.06 ± 4.56	38.4 - 52.9	60.83 ± 8.88	49.8 - 80.0	52.10 ± 5.46	44.3 - 57.0	[illegible]35.0	93.52 ± 2.93	[illegible]9.7 - 99.6
FEL	16.48 ± 1.41	14.4 - 19.7	15.49 ± [illegible].02	13.8 - 17.0	20.[illegible] ± 3.[illegible]8	16.8 - 25.9	16.70 ± 0.57	16.3 - 17.5	45.9	28.06 ± 3.44	23.0 - 32.[illegible]
TL	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FOL	18.24 ± 1.40	14.0 - 20.4	[illegible]4.74 ± [illegible].02	13.7 - 16.3	20.03 ± 3.09	14.4 - 25.6	17.23 ± 0.94	15.9 - [illegible]7.9	41.4	[illegible]	26.9 - 35.[illegible]
T1	6.49 ± 0.85	5.1 - 9.[illegible]	5.3[illegible] ± 0.90	4.4 - 7.1	7.46 ± [illegible]	4.0 - 10.9	6.73 ± 1.42	5.4 - 8.7	15.8	[illegible] ± 1.6[illegible]	[illegible]
T2	[illegible]	[illegible]	7.40 ± 0.91	[illegible]	[illegible]	[illegible]	8.98 ± [illegible]	[illegible]	[illegible]	15.9[illegible] ± 1.93	[illegible]
T3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
T4	14.65 ± 1.24	11.9 - 17.3	1[illegible].86 ± [illegible]	10.8 - 14.2	16.73 ± 3.[illegible]	12.[illegible] - 22.0	14.03 ± 1.39	12.8 - 16.0	36.0	[illegible]	[illegible]
T5	12.30 ± 1.21	10.3 - 14.5	10.20 ± 0.94	9.3 - 12.[illegible]	14.[illegible] ± 2.44	10.2 - 19.7	12.50 ± 1.34	11.3 - 13.9	29.7	[illegible]	[illegible]

Ecology. – Males were calling while floating among rice plants (fig. 5B). The calling males were observed in the middle portion of paddy fields without exception. On the banks of the same paddy fields, *Fejervarya granosa* Kuramoto et al., 2007 and *F. caperata* were actively calling. We could not collect females in paddy fields where males were calling.

Distribution. – Presently known only from the type locality, Mudigere.

Etymology. – Specific name was derived from the name of type locality, Mudigere. It is an invariable name in apposition to the generic name.

DNA sequence data for holotype. – Accession numbers are AB377110 and AB377109 for mitochondrial 12S and 16S rRNA genes, respectively (07-21 in fig. 1).

MORPHOLOGICAL COMPARISONS BETWEEN *EUPHLYCTIS* TAXA FROM KARNATAKA

As shown in tab. 1, *Euphlyctis aloysii* and *E. mudigere* are distinctly smaller than *E. hexadactylus*. Ranges of SVL of *E. aloysii* females and *E. mudigere* males do not overlap with those of *E. hexadactylus*. The snout-vent length of *E. aloysii* females is significantly smaller than that of *E. cyanophlyctis* females (U = 107, P = 0.035), whereas no significant difference was obtained between males of *E. mudigere* and *E. cyanophlyctis* (U = 5, P = 0.089). Fairly distinct large dark blotches on the dorsum of female *E. aloysii* were not observed in

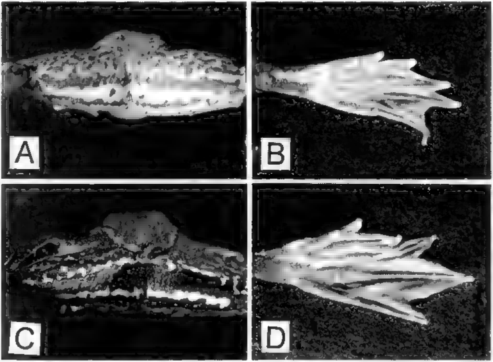


Fig. 7. Posterior side of thigh and foot of *E. cyanophlyctis* (RBRL 05070921, ♀ from Mudigere) (A, B) and those of *E. hexadactylus* (RBRL 06071903, ♂ from Adyar) (C, D)

E. hexadactylus and *E. cyanophlyctis*. Vomerine teeth of *E. hexadactylus* are distinct, forming two highly elevated oblique lines between choanae. In *E. cyanophlyctis*, subarticular tubercles are distinct in contrast to the indistinct tubercles of *E. aloysii* and *E. mudigere*. The mid-dorsal stripe is absent in *E. mudigere* and *E. cyanophlyctis*.

As a whole, *E. aloysii* and *E. mudigere* resemble *E. hexadactylus* and *E. cyanophlyctis*, respectively. However, large dark brown markings like those on the dorsum of *E. aloysii* were never observed in *E. hexadactylus* or any other *Euphlyctis* species. These markings were very conspicuous in specimens which died accidentally during transportation (RBRL 04070601, 04070602). The stripe pattern on the posterior side of the thigh of *E. hexadactylus* differs from that of *E. aloysii* consisting of two thinner white stripes and a much thicker black stripe between the two white stripes (fig. 7C). The web of *E. hexadactylus* is sharply incised as in *E. aloysii* (fig. 7D). The thigh stripe pattern of *E. mudigere* is similar to that of *E. cyanophlyctis* (fig. 7A), and the web is not deeply incised in both species (fig. 7B). The dorsal surface is densely covered with small granular tubercles in *E. cyanophlyctis*, whereas the granules are rather scarce in *E. mudigere*.

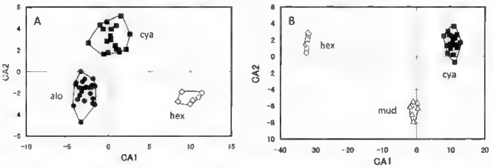
Euphlyctis aloysii was separated clearly from *E. hexadactylus* and *E. cyanophlyctis* by canonical discriminant analysis using measurements (fig. 8A). The statistics for discriminant

Table 2. – Statistics obtained from the discriminant analyses using measurements of five *Euphlyctis* species. Abbreviations: *alo*, *E. aloysii*; *cya*, *E. cyanophlyctis*; *ehr*, *E. ehrenbergii*; *hex*, *E. hexadactylus*; *mud*, *E. mudigere*.

Species compared	Number of variables	Eigenvalue			Wilks' lambda (*P*)			Discriminant result (%)	Figure
		Function 1	Function 2	Function 3	Function 1 (or 1-2, 1-3)	Function 2 (or 2-3)	Function 3		
alo, cya, hex	24	17.277	6.448	–	0.007 (< 0.001)	0.134 (< 0.001)	–	100	8A
mud, cya, hex	24	285.730	12.187	–	0.000 (< 0.001)	0.076 (0.004)	–	100	8B
alo, mud	24	54.045	–	–	0.018 (< 0.001)	–	–	100	9A
alo, mud, cya, hex	24	14.013	5.147	1.888	0.004 (< 0.001)	0.056 (< 0.001)	0.346 (0.004)	100	9B
ehr, cya, hex	18	23.108	5.187	–	0.007 (< 0.001)	0.162 (0.034)	–	100	10A
ehr, cya, hex	14	15.105	3.311	–	0.014 (< 0.001)	0.232 (0.025)	–	100	10B

Table 3. – Mean, standard deviation and range in body ratios of four *Euphlyctis* species from southwestern Karnataka, India. See text for character abbreviations.

Ratio	*E. [illegible]* (n = 24)		*E. mudigere* (n = 7)		*E. [illegible]* (n = 9)		*E. [illegible]* (n = 6)	
	x ± s	min - max	x ± s	min - max	x ± s	min - max	x ± s	min - max
HL/SVL	0.289 ± 0.020	0.252 - 0.331	0.307 ± 0.034	0.261 - 0.348	0.315 ± 0.02	0.288 - 0.388	0.348 ± 0.030	0.307 - 0.385
HW/SVL	[illegible]	[illegible] - 0.368	[illegible]	0.331 - [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible] ± 0.006	0.057 - 0.075	0.060 ± 0.005	0.063 - 0.078	0.061 ± [illegible]	0.047 - 0.080	[illegible] ± 0.006	0.040 - 0.055
[illegible]	[illegible] ± 0.008	[illegible] - [illegible]	0.078 ± 0.026	0.051 - [illegible]	0.060 ± [illegible]	0.056 - [illegible]	0.092 ± 0.004	[illegible] - 0.096
[illegible]	[illegible] ± [illegible]	0.063 - 0.130	0.127 ± 0.023	0.078 - [illegible]	[illegible] ± 0.019	0.102 - 0.185	0.110 ± [illegible]	[illegible] - 0.119
[illegible]	[illegible] ± 0.009	[illegible] - 0.069	0.047 ± 0.007	0.037 - 0.055	0.046 ± 0.018	0.036 - 0.111	0.035 ± 0.005	0.027 - [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TD/SVL	0.092 ± 0.013	0.066 - 0.117	0.095 ± 0.026	0.043 - [illegible]	[illegible] ± 0.011	0.087 - 0.134	0.096 ± 0.014	0.075 - 0.113
[illegible]	0.241 ± 0.025	0.174 - [illegible]	0.219 ± 0.051	0.155 - 0.276	0.265 ± 0.022	0.215 - 0.312	0.221 ± 0.032	0.165 - 0.252
F1/SVL	0.146 ± 0.025	[illegible] - 0.188	0.152 ± 0.024	[illegible]	[illegible] ± 0.02	[illegible] - 0.230	0.156 ± [illegible]	0.132 - 0.171
F2/SVL	0.121 ± 0.013	0.100 - 0.150	0.123 ± 0.017	0.093 - [illegible]	[illegible] ± 0.028	[illegible] - 0.252	0.142 ± 0.020	0.127 - 0.180
F3/SVL	[illegible]	[illegible]	[illegible]	0.161 - [illegible]	[illegible]	[illegible]	[illegible] ± 0.009	[illegible]
F4/SVL	[illegible] ± 0.015	[illegible] - 0.164	0.127 ± 0.023	0.099 - [illegible]	[illegible] ± [illegible]	[illegible] - 0.239	0.149 ± [illegible]	0.133 - 0.162
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
FL/SVL	0.439 ± 0.026	0.404 - 0.497	0.490 ± [illegible]	0.457 - 0.516	0.484 ± [illegible]	0.428 - 0.564	0.467 ± 0.040	[illegible]
TL/SVL	0.470 ± 0.027	[illegible]	0.480 ± 0.026	0.450 - 0.516	[illegible] ± [illegible]	0.449 - 0.600	0.480 ± [illegible]	[illegible] - 0.494
FOL/SVL	0.487 ± 0.034	0.390 - 0.553	0.466 ± 0.025	0.425 - 0.497	0.485 ± [illegible]	0.411 - 0.615	0.499 ± 0.022	0.468 - [illegible]
T1/SVL	0.171 ± 0.023	0.140 - 0.237	0.168 ± 0.023	0.137 - 0.204	0.181 ± 0.031	0.121 - 0.258	0.192 ± [illegible]	0.175 - 0.220
T2/SVL	[illegible] ± 0.021	0.202 - [illegible]	0.233 ± 0.015	0.220 - 0.264	0.245 ± 0.034	0.196 - 0.365	0.249 ± [illegible]	0.230 - [illegible]
T3/SVL	0.319 ± 0.030	[illegible] - 0.367	[illegible] ± 0.019	0.298 - 0.351	0.338 ± [illegible]	[illegible]	0.341 ± [illegible]	[illegible]
T4/SVL	0.390 ± 0.023	0.336 - [illegible]	0.375 ± 0.027	0.348 - 0.411	0.401 ± [illegible]	[illegible] - 0.479	[illegible] ± 0.031	0.384 - [illegible]
T5/SVL	0.328 ± 0.026	[illegible] - 0.370	0.322 ± 0.019	[illegible] - 0.351	0.343 ± [illegible]	[illegible] - 0.403	[illegible] ± 0.021	0.300 - [illegible]
HL/HW	0.877 ± [illegible]	[illegible]	0.849 ± 0.098	[illegible] - 1.008	0.927 ± [illegible]	0.841 - [illegible]	[illegible] ± 0.088	0.897 - [illegible]
[illegible]	0.925 ± 0.170	0.633 - 1.273	[illegible]	0.479 - 2.000	0.952 ± [illegible]	0.645 - [illegible]	0.878 ± [illegible]	[illegible] - 1.059
TD/ED	[illegible]	0.650 - 1.606	0.773 ± [illegible]	0.560 - 0.971	0.890 ± [illegible]	0.656 - [illegible]	0.873 ± 0.092	0.733 - [illegible]
[illegible]	1.569 ± 0.309	1.045 - 2.000	1.505 ± 0.279	[illegible] - 1.917	1.451 ± 0.406	0.500 - 2.700	1.465 ± 0.362	1.029 - 2.067
[illegible]	1.600 ± [illegible]	0.955 - [illegible]	1.466 ± 0.477	0.875 - 1.917	1.515 ± 0.488	0.548 - [illegible]	1.607 ± 0.224	[illegible]
F1/F2	[illegible]	0.922 - [illegible]	1.240 ± 0.089	[illegible] - 1.326	1.295 ± [illegible]	0.705 - [illegible]	[illegible]	0.855 - [illegible]
[illegible]	1.071 ± 0.072	0.865 - [illegible]	0.981 ± 0.043	0.910 - 1.053	1.036 ± 0.062	0.925 - 1.150	1.034 ± 0.070	[illegible]
[illegible]	1.109 ± 0.062	0.966 - 1.209	0.953 ± [illegible]	0.885 - 0.994	[illegible] ± 0.067	0.857 - [illegible]	1.076 ± [illegible]	0.902 - 1.20



ig. 8 Scatter plot of individual score of canonical discriminant function 1 (CA1) and 2 (CA2) for *E. aloysii*, *E. cyanophlyctis*, and *E. hexadactylus* (A) and that for *E. mudigere*, *E. cyanophlyctis*, and *E. hexadactylus* (B)

nalysis are shown in tab. 2. The standardized discriminant coefficients were large (in bsolute value) in SVL, HLL and HL for function 1 and in SVL, T4, F1 and F2 for function ' In discriminant analysis using ratios relative to SVL (HL/SVL, HW/SVL, etc.), the listribution pattern of individual scores was nearly the same as in the analysis using neasurements. Mann-Whitney *U* tests showed that nine and 13 body ratios differed ignificantly ($P < 0.01$) between *E. aloysii* and *E. hexadactylus* and between *E. aloysii* nd *E. cyanophlyctis*, respectively (tab. 3-4). The head is smaller in *E. aloysii* than in *H. hexadactylus*, differences of both HL/SVL and HW/SVL of the two species being highly ignificant ($P < 0.01$). The eyelid width is larger and the hindlimb length is smaller, both elative to SVL, in *E. aloysii* than in *E. hexadactylus* ($P < 0.01$). *Euphlyctis aloysii* differs ignificantly from *E. cyanophlyctis* ($P < 0.01$), having a smaller head length, smaller eye liameter, tympanum diameter, femur length and tibia length, all relative to SVL. The ratio IL/HW is significantly smaller, and FOL/FEL is significantly larger in *E. aloysii* than in *E. cyanophlyctis*.

Euphlyctis mudigere was also clearly separated from *E. cyanophlyctis* and *E. hexadactylus* y discriminant analysis (fig. 8B, tab. 2). The standardized coefficients of discriminant unctions revealed that HW, T4, T2 and F3 contributed more to function 1 and T4, TIL nd FOL contributed more to function 2 than the other measurements. Only two and ne body ratios were significantly different ($P < 0.01$) between *E. mudigere* and *E. cyanophlyctis* and between *E. mudigere* and *E. hexadactylus* respectively (tab. 3-4). The ratios 1/SVL and F2/SVL were significantly smaller in *E. mudigere* than in *E. cyanophlyctis* $P < 0.01$), and N-N/SVL was significantly larger in *E. mudigere* than in *E. hexadactylus* $P < 0.01$). Fingers and toes were shorter in *E. mudigere* than in *E. cyanophlyctis* and *E. hexadactylus*.

Discriminant analysis clearly separated *E. mudigere* from *E. aloysii* (fig. 9A, tab. 2). The tandardized coefficients of the discriminant function were large (in absolute value) in N-E, 14, F1 and SVL. Mann-Whitney *U* tests revealed that the ratios HW/SVL, FEL/SVL and TIL/ EL were significantly larger ($P < 0.01$) and FOL/FEL was significantly smaller ($P < 0.01$) in *E. mudigere* than in *E. aloysii* (tab. 3-4).

Table 4. – Results of Mann Whitney U test between body ratios of *E. aloysii* (*alo*), *E. mudigere* (*mud*), *E. cyanophlyctis* (*cya*) and *E. hexadactylus* (*hex*). U and P values are given. Symbols * and ** indicate the 5 % and 1 % significance levels, respectively.

Ratio	*alo* vs. *mud*		*alo* vs. *cya*		*alo* vs. *hex*		*mud* vs. *cya*		*mud* vs. *hex*		*cya* vs. *hex*	
	U	*P*	*U*	*P*	*U*	*P*	*U*	*P*	*U*	*P*	*U*	*P*
HL/SVL	69	0.478	88	0.001 **	6	0.001 **	52	0.402	7	0.046 *	19	[illegible] *
HW/SVL	23	0.004 **	158	0.087	18	0.005 **	37	0.088	20	0.886	32	[illegible]
S-N/SVL	56	0.186	106	0.003 **	19	0.006 **	62	0.795	20	0.886	37	[illegible]
N-N/SVL	60	0.257	148	0.350	0	0.000 **	34	0.060	0	0.003 **	23	0.031 *
N-E/SVL	81	0.887	103	0.002 **	4	0.000 **	55	0.506	12	0.199	13	0.005 **
ED/SVL	30	0.011 *	33	0.000 **	37	0.070	66	0.977	11	0.153	20	0.019 *
E-E/SVL	52	[illegible]	227	0.980	34	0.049 *	53	0.435	4.5	[illegible] *	29	0.075
ELW/SVL	79	0.813	173.5	0.183	14	0.003 **	49	0.312	7	0.046 *	27	0.056
TD/SVL	63	0.321	54	0.000 **	60	0.534	39	0.112	19	0.775	19	0.016 *
HAL/SVL	67	0.422	100	0.002 **	46	0.178	30	0.035 *	21	1.000	5	0.001 **
F1/SVL	70	0.508	32	0.000 **	52	0.300	11	0.001 **	19	0.775	8	0.002 **
F2/SVL	73	0.603	37	0.000 **	20	0.007 **	16	0.004 **	7	0.046 *	36	[illegible]
F3/SVL	52	[illegible]	[illegible]	0.004 **	43	0.133	54	0.470	20	0.886	43	0.373
F4/SVL	76	0.705	181	0.250	26	0.017 *	51	0.370	9	0.086	35	0.162
HLL/SVL	80	0.850	139	0.030 *	14	0.003 **	48	0.285	11	0.153	38	[illegible]
FEL/SVL	9	0.000 **	69.5	0.000 **	39	0.087	55	0.506	14	0.317	44	[illegible]
TIL/SVL	66	0.395	106	0.003 **	49	0.233	45	0.214	16	0.475	31	[illegible]
FOL/SVL	50	[illegible]	197	0.448	56	0.407	53	0.435	7	0.046 *	39	[illegible]
T1/SVL	73	0.603	193	0.392	31	0.034 *	44	0.193	8	0.063	33	[illegible]
T2/SVL	62	0.299	224	0.922	55	0.378	45	0.214	9	0.086	43	0.373
T3/SVL	76	0.705	163	0.112	41	[illegible]	50	0.340	12	0.199	50	0.656
T4/SVL	56	0.186	211	0.678	35	0.055	41	0.140	9	0.086	39	0.252
T5/SVL	70	0.508	177	0.212	70	0.917	45	0.214	18	0.668	40	[illegible]
HL/HW	60	0.257	115	0.006 **	17	0.004 **	31	0.040 *	5	0.022 *	39	[illegible]
S-N/N-E	53	0.143	211.5	0.686	63.5	0.659	46	0.236	12	0.199	46.5	[illegible]
TD/FD	50.5	0.141	218	0.807	67.5	0.815	42	0.157	14	0.317	48.5	0.588
N-N/E-E	72.5	0.587	183.5	0.276	58.5	0.484	61.5	0.773	19	0.775	55.5	0.924
ELW/E-F	72.5	0.587	217	0.788	71.5	0.979	58.5	0.644	17	0.568	54.5	0.874
F1/F2	68.5	0.464	161.5	[illegible]	50	0.254	51	0.370	11	0.153	24.5	0.039 *
TIL/FEL	19	0.002 **	154.5	0.072	44	[illegible]	32	0.046 *	10	0.116	53	[illegible]
FOL/FEL	2	0.000 **	49	0.000 **	60	0.534	34	0.060	6	0.032 *	28	0.065

Finally, all four *Euphlyctis* species from Karnataka were separated by discriminant analysis (fig. 9B; tab. 2). The standardized coefficients of discriminant functions were large (in absolute value) in SVL, HLL, FEL and HW for function 1, in SVL, F1, T4 and F2 for function 2, and in FOL, SVL, T5 and T4 for function 3. Although the plot range of *E. mudigere* slightly overlapped with those of *E. aloysii* and *E. cyanophlyctis* in fig. 9B, *E. mudigere* was clearly separated along the third axis for discriminant function 3, scores for function 3 being from 2.431 to 4.263 for *E. mudigere*, from −2.931 to 1.016 for *E. aloysii* and from −2.629 to 1.374 for *E. cyanophlyctis*.

Discussion

Many lines of evidence suggest the existence of a considerable amount of genetic divergence between populations of the wide-ranging *E. cyanophlyctis* populations. Khan (1997) described a subspecies of *E. cyanophlyctis* from the northwestern highlands of Pakistan as *E. cyanophlyctis microspinulata*. Dutta (1997) considered *E. cyanophlyctis seistanica*, described from Iran by Nikolskii (1900) as a variety, as a valid subspecies. Alam et al. (2008) clarified that each of the *E. cyanophlyctis* populations from southwestern India, Bangladesh and Sri Lanka constitutes distinct clusters in the phylogenetic tree constructed on the basis of mtDNA sequence data. Remarkable acoustic differences between southwestern and northeastern populations of Indian *E. cyanophlyctis* (Roy & Elepfandt, 1993; Kuramoto &

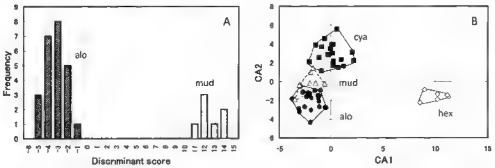


Fig. 9. Distribution of discriminant scores of *E. aloysii* and *E. mudigere* (A) and scatter plot of individual score of canonical discriminant function 1 (CA1) and 2 (CA2) for *E. aloysii*, *E. mudigere*, *E. cyanophlyctis* and *E. hexadactylus* (B).

JOSHY, in press) may reflect genetic divergence between the two Indian populations. It seems highly probable that future studies will reveal the existence of several cryptic species allied to *E. cyanophlyctis*.

The type locality of *E. cyanophlyctis* (*Rana cyanophlyctis*) is probably Tranquebar (Tarangambadi) in east-central Tamil Nadu, India (BAUER, 1998). Although TIWARI (1991) regarded Kerala, most of Tamil Nadu and southwestern Karnataka as belonging to the Malabar faunal province in the Ceylonese sub-region of the Oriental faunal region, this does not mean the genetic identity of *E. cyanophlyctis* occurs there. Further molecular phylogenetic studies are needed to clarify the relationship of *E. cyanophlyctis* from Karnataka.

The distribution range of *E. hexadactylus* is confined to India, Bangladesh and Sri Lanka. The type locality of this species is south India (FROST, 1985). Although *E. hexadactylus* was reported to have a white or pale yellow venter (DUTTA & MANAMENDRA-ARACHCHI, 1996; CHANDA, 2002; DANIEL, 2002; DANIELS, 2005), all six specimens from Mangalore have a finely mottled pattern on the venter and lower side of the thigh, which is never observed in *E. aloysii*, *E. mudigere* and *E. cyanophlyctis*. The rather heavily mottled underside observed in the *E. hexadactylus* specimens examined in this study indicates genetic differentiation within this species. Thus, the taxonomic situation of *E. hexadactylus* from Karnataka is similar to that of *E. cyanophlyctis* mentioned above.

Euphlyctis ehrenbergii had long been synonymized with *E. cyanophlyctis* and was resurrected by DUBOIS (1981). This species is relatively large in size and has a uniformly greenish dorsum (LEVITON et al., 1992; KHAN, 1997), resembling *E. hexadactylus*. BOULENGER (1920) gave measurements for eight specimens of *E. ehrenbergii* (as *Rana cyanophlyctis* from Saudi Arabia and Yemen), and this species was clearly separated from *E. cyanophlyctis* ($n = 9$) and *E. hexadactylus* ($n = 8$) both from southern India and Sri Lanka by discriminant analysis using his measurements (fig. 10A; tab. 2). Comparisons for body ratios revealed that HL/SVL and F1/F2 of *E. ehrenbergii* were greater ($P < 0.01$) than those of *E. cyanophlyctis* and F1/SVL, F4/SVL, TIL/SVL and TIL/FEL were larger ($P < 0.01$) and F1/F2 was smaller ($P < 0.01$) than those of *E. hexadactylus*. These comparisons give morphometric bases for the specific distinctness of *E. ehrenbergii*.

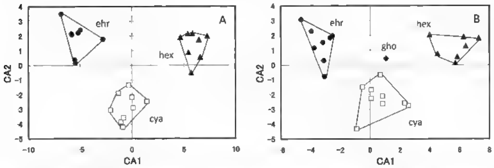


Fig. 10. - Scatter plot of individual score of canonical discriminant function 1 (CA1) and 2 (CA2) for *E. cyanophlyctis*, *E. hexadactylus*, both from south India and Sri Lanka, and *E. ehrenbergii* from Saudi Arabia and Yemen (A). On the scatter plot for the above three species (based on lower number of variables), the score of *E. ghoshi* calculated from the coefficients for the three species is plotted (B). Data from BOULENGER (1920) and CHANDA (1990).

ROY & ELEPFANDT (1993) revealed acoustic differences between *E. ehrenbergii* and *E. cyanophlyctis*. Acoustic features of *E. hexadactylus* were analyzed by KURAMOTO & JOSHY (in press), which seemed rather similar to *E. ehrenbergii* than to *E. cyanophlyctis*. The *E. hexadactylus* population from Bangladesh was proved to belong to a new undescribed taxon by molecular evidence (ALAM et al., 2008).

CHANDA (1990), in describing *E. ghoshi* (as *Rana ghoshi*), suggested the close relationships of *E. ghoshi* with *E. cyanophlyctis* (as *Rana cyanophlyctis*), *Lankanectes corrugatus* (Peters, 1863) (as *Rana corrugata*) and *Chrysopaa sternosignata* (Murray, 1885) (as *Rana sternosignata*). Each of these genera belongs in a different tribe in the subfamily Dicroglossinae or different subfamily in the Ranidae (DUBOIS, 2005), and the phylogenetic relationship of *E. ghoshi* must wait for future studies. CHANDA (1990) gave measurements for the holotype of *E. ghoshi*. When the discriminant scores for this *E. ghoshi* specimen were calculated using the coefficients of canonical discriminant functions for *E. ehrenbergii*, *E. cyanophlyctis* and *E. hexadactylus* (all data from BOULENGER, 1920, as in fig. 10A, except F4, TIL, FOL and T5 which were lacking for *E. ghoshi*, and forelimb length which was measured apparently in different ways by BOULENGER, 1920 and by CHANDA, 1990), the plot was separated from the ranges of the other three species (fig. 10B, tab. 2). In view of the fact that the ratios snout-length/SVL (15.0), ED/SVL (13.3), and E-E/SVL (6.7) of *E. ghoshi* were larger and HLL/SVL (126.7), T3/SVL (24.2) and TD/ED (0.5) were smaller than the maximum and minimum values, respectively, for *E. ehrenbergii*, *E. cyanophlyctis* and *E. hexadactylus*, *E. ghoshi* seemed to be related rather remotely with the other three *Euphlyctis* species. The snout of *E. ghoshi* (fig. 1 in CHANDA, 1990) was round which is unlike the rather pointed snouts of congeners.

The genus *Euphlyctis* has many taxonomic problems to be solved as mentioned above, and future studies may reveal several new cryptic species, as in "*Fejervarya limnocharis*", which was once considered to have an extensive distribution range and recently was split into many species (DUBOIS & OHLER, 2000; FEI et al., 2002; KURAMOTO et al., 2007; MATSUI et al., 2007).

ACKNOWLEDGEMENTS

We thank Rev Fr Swebert D'Silva, the Principal of St Aloysius College, and Rev Fr Leo D'Souza for support and facilities to carry out the research, and J. D'Souza and K. G. Yogish for aid in the field. SHJ thanks Jesuit Educational Society for the support, and Melwyn Sequeira and Santhosh Wilson for aid in the laboratory.

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